Seroprevalence of Human T-cell Lymphotrophic Virus Types I/II (HTLV-I/II) among Blood Donors in a Tertiary Hospital in Oman

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Abstract

Objectives: Routine blood donor screening of Human T-cell Lymphotropic Virus (HTLV) has been practiced in Oman since 2017. There is limited data on HTLV seroprevalence among Omanis. This study aims to determine the seroprevalence for HTLV-I/II among blood donors attending a hospital-based blood bank to assess the need for a universal versus targeted screening.

Methods: A retrospective review of blood donors' results attending a hospital blood bank between January 2017 and February 2020 was performed. Blood samples were screened for HTLV-I/II antibodies using ARCHITECT i2000SR. Reactive samples were further tested by immunoblot assay (MP Diagnostics HTLV Blot 2.4). Age, gender and nationality were assessed. All components manufactured at the blood bank are leukoreduced pre-storage.

Results: A total 24,469 first-time blood donors were screened for HTLV antibodies. Most of the participants were male (n=22,186, 90.7%), and the majority were Omani (n=22,711, 92.8%). The age range was between 18 to 64 years with a median of 32 years. The seroreactivity rate was 0.176% (43/24,469) (95% CI: 0.12-0.23%). Confirmatory testing by immunoblot revealed 3 indeterminate results (7%), of which 2 were Omani and 1 non-Omani donors, and the remaining 40 sero-reactive donors tested negative.

Conclusions: Our study revealed zero seroprevalence of confirmed HTLV among blood donors. The continuation of universal screening of first-time donors is a standard of care. In the presence of universal leukoreduction at SQUH and very low risk of HTLV in Oman's population, the need for screening regular donors can be re-considered if these findings were confirmed on a larger scale involving other blood banks in Oman.

Keywords: Human T-cell lymphotropic virus types I and II (HTLV-I and -II); blood donors; seroprevalence; Oman.

Introduction

Human T-cell Lymphotropic Virus (HTLV) is the first human retrovirus to be discovered in 1979 that belongs to the Retroviridae family.[1] The most prevalent and pathogenic HTLV types are types 1 and 2, while little is known about the recently discovered types 3 and 4, isolated in few cases in Africa.[2] HTLV type 1 (HTLV-I) was identified initially from an American patient with cutaneous T-cell lymphoma, while HTLV type 2 (HTLV-II) was isolated two years later in a patient with variant T-cell hairy cell leukemia.[3] These two viruses vary in their epidemiology and disease

profile. The most serious clinical diseases associated with HTLV-I are adult T-cell leukaemia/lymphoma (ATLL) and HTLV-I associated myelopathy (HAM).[1] In addition, other diseases have been linked to HTLV-I such as infective dermatitis, uveitis and polymyositis.[1,4]

The interaction of HTLV-I with host immune response is an important factor in the pathogenesis of HTLV-I associated diseases. The virus alters Toll-like receptor-4 (TLR4) signaling pathway,[5] which plays a significant role in the host innate response against bacterial infections,[6] and stimulate secretion of interlukin (IL)-10 that promotes cell proliferation in HTLV-I infected cells through the STAT3 and IRF4 pathways.[7] Additionally, transforming growth factor (TGF)- β1 is another important T helper 3 (TH3) immune suppressor cytokine that promotes B-cell and T-helper cell interaction.[8]

Programmed death 1 (PD-1) and programmed death ligand 1(PD-L1) pathway, which induces apoptosis, is overexpressed in HTLV-I specific $CD8^+$ T-cells[9] leading to exhaustion of T-cells and chronic viral infection and thus therapeutic failures.[10] It was found that HTLV-I infected patients are more susceptible to Tuberculosis (TB) particularly in endemic areas due to a dysregulated immune system response.[11] Also, HTLV-I infection was found to worsen the clinical course of HCV infection, contributing to the development of Hepatocellular Carcinoma (HCC) in these patients.[12]

Approximately 5-10 million individuals in the world are infected with HTLV-I.[13] It is distributed worldwide and found to be endemic in southwestern Japan,[2] Taiwan,[13] Sub-Saharan African countries,[3] the Caribbean basin, and South America.[2] The seroprevalence in these areas is high and reported as high as 14% of the tested population.[3,14] In these endemic areas, the rate of sero-positivity increases as age advances and can reach as high as 30 to 40% in individuals above 50 years of age.[1] In addition, HTLV-I is endemic in certain regions in the Middle East and India.[15] A discrepancy in the prevalence of HTLV-I was found among different regions in Iran ranging from 0 to 2.3%.[13] In Pakistan, the prevalence rate of HTLV-I among blood donors is found to be 0.19%, whereas it was found to be 0.0021% in Turkey.[5] In Saudi Arabia, several studies were done to assess the prevalence of the disease and showed prevalence rates ranging from 0 to 0.006%.[2,16]

HTLV-II has a restricted geographical distribution when compared to HTLV-I. It is endemic in Europe and North America, particularly among intravenous drug users.[1] It is less pathogenic than HTLV-I; therefore, it's association to clinical disease is less evident. Nevertheless, it has been linked to chronic pulmonary diseases and neurological disorders.[1] Both HTLV-I and II are transmitted through different routes mainly through sexual transmission, vertical transmission, breastfeeding and parenteral transmission through transfusion or sharing infected needles.[17] If a blood recipient got a contaminated blood product with HTLV-I in an endemic area, the likelihood of the recipient's seroconversion is 40-60% within 60 days after transfusion.[5]

Transfusion is one of the major routes of HTLV-I transmission, even in non-endemic areas. Since there is a high rate of seroconversion following transfusion of contaminated blood products, especially in endemic areas, the risk of transmissible HTLV infection by blood donors was considered. As per the World Health Organization (WHO) recommendations, HTLV I/II screening should be considered prior to the release of blood units.[18] The WHO recommends screening of blood donors for HTLV-I based on each country's epidemiological evidence. In addition, non-endemic areas can get migrants from HTLV endemic areas and can be potential donors who can pose a risk of HTLV transmission via blood transfusion.

Some endemic countries have implemented donor screening for HTLV since long time, for instance; Japan mandated this protocol since 1986, followed by the United States and the French Caribbean in 1989.[19] Practice is variable in other countries including Europe and the Middle East. For instance, Iran started the screening program in 1994, while other countries in the Gulf like Saudi Arabia are still reviewing the need for stopping mandatory universal blood screening for HTLV infection due the very low prevalence based on their studies.[3] To the best of our knowledge, there is only one study done to assess HTLV seroprevalence in Oman. The study was conducted in 1997 and showed HTLV seroprevalence of 0.6% among blood donors (9 out of 1586) using enzyme immunoassay (EIA) and confirmatory test showed indeterminate result in 6 out of 9 tested reactive by screening (0.4%).[20] However, the sample size was small, the methodology used was not detailed. There were no other studies that were published since then.

The Sultan Qaboos University Hospital (SQUH) blood bank is an independent blood bank facility located in a tertiary care reference university hospital in Muscat. The blood bank performs donor collection, screening, testing and blood component manufacturing. All cellular blood components are leukoreduced. Donor HTLV screening has been instated at SQUH in January 2017. The aim of this study is to determine the seroprevalence of HTLV-I/II among blood donors attending the SQUH Blood Bank, to assess the need for universal versus targeted screening of blood donors in Oman.

Methods

This is a retrospective cross-sectional study assessing HTLV-I/II serological results of blood donors donating blood at the SQUH Blood Bank. Records of all donors donating blood during the period between January 2017 and February 2020 were reviewed. Data were collected from the hospital information system. Variables assessed included donor's demographics (gender, age, nationality and place of residency), the results of HTLV-I/II screening and any confirmatory test done.

Donor's blood samples are screened for HTLV-I/ II antibodies using Chemiluminescent Microparticle Immunoassay (CMIA, ARCHITECT i2000SR, Abbott Diagnostics, U.S) according to the manufacturer's instructions. The test sensitivity and specificity are 100% and ≥ 99.5%, respectively. Blood samples are considered reactive for HTLV-I/II antibodies if the signal/cutoff (S/CO) ratio is ≥ 1.0. All initial reactive samples get repeated in duplicate using the same assay. The repeat reactive samples were followed by confirmation testing using HTLV- I/II Immunoblot assay (MP Diagnostics HTLV Blot 2.4, MP Biomedicals Asia Pacific Pte. Ltd), and interpreted according to manufacturer's instructions. The diagnosis of HTLV infection is made when the test is reactive in both CMIA and immunoblot testing. In the event of reactive HTLV screening test, the donor will be temporary deferred from donation till the results of further confirmatory testing are released. Donors with confirmed positive or indeterminate results, are referred to infectious diseases team for counselling and further management.

The minimum required calculated sample size was around 23,000 blood donors. The following assumptions were set to calculate this sample size are the previous local prevalence of HTLV-I in Oman which is 0.6%,[20] precision of 0.1% and confidence level of 95%. Seroprevalence data were calculated from all blood donor test results. Statistical analysis was performed using_SPSS version 22 software. Categorized variables were described as percentages. Prevalence was calculated as a percentage with 95% confidence interval.

Ethical approval was obtained from the Medical Research Ethics Committee at the College of Medicine and Health Sciences, the Sultan Qaboos University (MREC#1786).

Results

A total of 24,469 first-time blood donors were included in the study. The donor age range was between 18 to 64 years, with a median of 32 years. The majority of the donors were males (22,186, 90.7%) and were Omanis (n=22,718, 92.8%). The demographic characteristics of the blood donors are summarized in Table 1.

Table 1: Demographic characteristics of the blood donors.

Variable	Number	Percentage %
Gender		
Male	22,186	90.7
Female	2,282	9.3
Age group, years		

18-20	2,794	11.4
21-30	11,107	45.4
31-40	7,569	31
41-50	2,564	10.4
>50	436	1.8
Nationality		
Omani	22,718	92.8
Non-Omani	1,751	7.2

Out of the 24,469 screened blood donors, 43 were repeatedly reactive for HTLV by CMIA (0.176%, 95% CI: 0.12-0.23). The S/CO ratio of reactive samples ranged from 1.00 to 34.5, with a median of 1.90 (Table 2). The majority of the sero-reactive donors were males (39/43, 91%). The age range was between 19 to 48 years, with a median of 27 years. Most of these donors were Omani (n=39, 90.7%), while four were non-Omani (9.3%) (Table 2).

Table 2: Demographic characteristic of HTLV-I/II sero-reactive blood donors.

Variable	Number	Percentage %
Gender		
Male	39	90.7
Female	4	9.3
Age group, years		
19-25	19	44.2
26-35	18	41.9
36-48	6	13.9
Nationality		
Omani	39	90.6
Non-Omani	4	9.3

Repeatedly reactive samples were further tested by an immunoblot confirmatory assay. Out of the 43 reactive screening samples, 40 (93%) were negative, and 3 (7%) were indeterminate by immunoblot (Table 3). The indeterminate group consisted of one Omani male, one Omani female, and one non-Omani male donor. Two indeterminate profiles showed only one envelope band (GD21/rgp46-II), and one showed two envelope bands (GD21 and rgp46-II), with CMIA S/CO ratios of 1.09,1.55 and 2.33, respectively. Considering there is no confirmed HTLV I/II infection in this cohort, the overall HTLV-I/II virus seroprevalence is 0% among all donors.

Table 3: HTLV-I/II screening and Immunoblot results.

HTLV-I/II Screening		HTLV-I/II Western blot		
S/CO value	**Number**	**Positive**	**Indeterminate**	**Negative**
1-5	38	-	3	35
5-10	3	-	-	3
>10	2	-	-	2

Discussion

This is the largest study to determine the seroprevalence of HTLV-I/II infection among blood donors in Oman. The study revealed a 0% seroprevalence of confirmed HTLV-I/II infection. None of the seroreactive Omani blood donors on initial screening was confirmed positive by the immunoblot, with the exception of the three indeterminate results. The indeterminate results could be attributed to several factors like cross-reactivity to other retroviruses, defective HTLV-I/II, antibody-reaction to Plasmodium *falciparum* or a delayed seroconversion.[21] In order to clarify those indeterminate results, molecular testing by polymerase chain reaction (PCR) is needed and can aid in the confirmation of HTLV I/II infection for the indeterminate cases. This test is however not available in our study center.

We report 0% HTLV-I/II seroprevalence, unlike the previously reported data by Knox-Macaulay et.al. from 1997, which showed HTLV-I seropositivity of 0.6% (9 out of 1586) by EIA, 0.4% (6 indeterminate results out of 9) by immunoblot.[20] None of these cases were confirmed as positive. The discrepancy in the reactivity rate could be explained by the difference in the sample size between the two studies, and the substantial improvement in the overall performance of the current screening and confirmatory assays compared to those used previously. The reactivity rate of CMIA screening of 0.176% could also be attributed to the high sensitivity offered by the employed kit in this study, leading to false-positive results.

Overall, most studies that have been conducted in the Arabian Peninsula reported a very low or zero seroprevalence rate of HTLV-I/II infection. In numerous studies involving blood donors in studies of HTLV-I/II seroprevalence, sero-reactive group consisted mainly of males which was explained by the higher proportion of male donors than female in these studies[2,3,5,8,15]. Saudi Arabia is one of the countries which extensively studied the prevalence of HTLV-I/II among blood donors. A study that included 107,419 blood donors revealed CMIA reactivity of 0.088%, and none was confirmed by immunoblot.[3] Similarly, several other reports in different tertiary hospitals in Saudi Arabia showed zero prevalence of HTLV-I/II,[2,22] which was in agreement with our study findings. On the other hand, a study in Kuwait among 46,039 volunteer blood donors showed an overall frequency of HTLV-I positive donations of 1:7,212 among Kuwaiti nationals and 1: 1,500 among Indians justifying compulsory screening of donated blood HTLV-I. Interestingly, an Omani male in this study was confirmed positive for HTLV-I by Western blot out of 21 other positive donors.[16]

HTLV-I infection is endemic in northeastern Iran (Mashhad) where the population prevalence is 2.12%.[23] A study evaluated 1,864,489 blood donations of seven Iranian blood transfusion centers for HTLV-I/II infection during five-years, revealed an overall HTLV-I prevalence of 0.098% with a descending trend over the years. This was attributed to the improved donor selection process, and the permanent deferral of previously seropositive donors from donating blood.[13] Seroprevalence of HTLV-I/II among blood donors in Lebanon and Turkey, were reported at 0.028% and 0%, respectively.[24,25] No publications on the prevalence rate of HTLV-I/II could be found in other countries in the region.

Our study has some limitations. This study was conducted in a single center where blood donors are mainly from Muscat Governorate. Potential differences in seroprevalence in donors from different regions in the country could not be assessed. The ethnicity of the non-Omani donors in our study cohort was not assessed. We utilized a convenient healthy population to study a prevalence rate of viral infection, which can introduce a selection bias and might not truly reflect the actual prevalence of the general population which include other high-risk groups. However, this is the largest study to assess the seroprevalence of HTLV-I/II among Omani donors. In order to determine a precise seroprevalence of HTLV-I/II in Oman, a large multi-center study including other blood banks in the country is needed. In addition, a confirmatory assay such as PCR for proviral DNA is needed to confirm the significance of the indeterminate results.

In conclusion, this study identified zero seroprevalence of confirmed HTLV I/II infection among blood donors in our center. This suggests that HTLV might not be of a public health concern in Oman, however, continuation of universal screening of first-time donors is a standard of care. In addition, HTLV seroprevalence need to be monitored as it can change with time. In the presence of universal leukoreduction at SQUH and very low risk of HTLV in Oman's population, we might consider stopping screening regular donors after confirmation of our findings on a larger scale involving other blood banks in other regions in Oman.

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